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Nanoscopic Portrait of an Amyloidogenic Pathway Visualized through Tip-Enhanced Raman Spectroscopy

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ABSTRACT: Inroads into understanding the process by which amyloid proteins become toxic have been hampered by the lack of experimental techniques that adequately resolve the process. Recently, tip-enhanced Raman spectroscopy, with its unique capability to spectroscopically image and chemically identify reaction mixtures with nanoscale precision, was used to obtain a high-resolution roadmap of the soluble-to-toxic conversion of amyloid beta. This technique opens the door for studying the toxic aggregation pathways of other amyloid proteins and spurs efforts devoted to prophylactic and therapeutic intervention in neurodegenerative and protein-misfolding-related disorders.

KEYWORDS: *Amyloidogenesis, amyloid beta, tip-enhanced Raman spectroscopy, toxic conversion, oligomers, protofibrils, fibrils*

The conversion of soluble amyloid proteins to their toxic oligomers and smaller aggregates, known as protofibrils, is a key event that transitions nontoxic proteins into pathogenic conformers (Scheme 1). It is a consequence associated with both the onset and progress of both neurodegenerative and non-neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), mutant Huntington's disease (mHTT), lysozyme amyloidosis, and type II diabetes. In these neuropathies, oligomers and protofibrils of the disease-specific amyloids, such as amyloid beta (Aβ), tau, α-synuclein, mutant Huntington protein, human lysozyme, and islet amyloid polypeptide (IAPP) dictate the severity of the disease in contrast to mature fibrils or plaque load. Therefore, a localized, chemical and morphological resolution (with nanometer precision) of the trajectory by which soluble, monomeric amyloids morph into their toxic forms is paramount to understanding not only how pathology onsets and progresses but also to leveraging prophylactic and therapeutic gains against their respective disorders.

Thus far, our understanding of the properties of amyloid proteins and their tendencies to self-assemble into toxic conformations is typically derived from (i) in vitro techniques that "artificially" stabilize intermediates, (ii) the use of chemical labeling that tends to limit conformational data to label-sensitive regions, (iii) spectroscopic assays that provide "signal averaged" outputs, or (iv) from simulation studies that have their own limitations. The inherent drawbacks in experimental and computational tools, coupled with the susceptibility of amyloidogenic trajectories to the study conditions (pH, temperature, buffer, molecular dynamics parameters), has, at times, resulted in conflicting literature about pathways to toxic conformations and, in other instances, provided a simplified, one-dimensional trajectory of the monomer → fibril transition (Scheme 1).

The true scenario may be more complex in that there may exist multiple pathways leading to the formation of oligomers and protofibrils from their respective, monomeric amyloid conformer. For example, heretofore undetected intermediates could populate the toxic conversion pathway in amyloids making the consumption of oligomers and protofibrils to higher-ordered aggregates a multistep process. The network of interactions between monomers, oligomers, protofibrils, and mature fibrils may also be populated with kinetically trapped species or dead-end conformers, both of which need to be identified as such to avoid false therapeutic targets. Hence, a high-resolution map that adequately distinguishes each unique conformer from another, a knowledge of the forward and reverse rate-constants pertaining to conformer interconversion, and an understanding of the impact that perturbants (pH, salt, temperature, mutations) may impose on not only the structures but also their rates of formation and consumption of the said intermediates remains the holy grail of amyloidogenesis.

In practice, our limited knowledge of the processes driving conversion of amyloid proteins to their toxic counterparts has hampered efforts to (i) fully understand the molecular basis for disease spread, (ii) determine the origins of cross-reactivity among amyloid proteins differing in sequence, and (iii) advance strategies that reduce toxic species burden and limit amyloid-driven pathology.

In a recent landmark study, Zenobi's laboratory used tip-enhanced Raman spectroscopy (TERS) to probe the aggregation trajectory of Aβ.[1] Tip-enhanced Raman spectroscopy, which combines scanning probe microscopy and Raman spectroscopy, is a label-free technique that can be performed in an ambient environment and in aqueous media. Hence, TERS is well-suited for simultaneously studying chemical composition, elementary dimensions, species architectures, process kinetics, and molecular dynamics of biological samples with localized nanoscale precision.[2−4]

The authors obtained a snapshot of the Aβ aggregation landscape 36 h after initiation of the process.[1] Conformers populating reaction mixture were selected for TERS/Raman-

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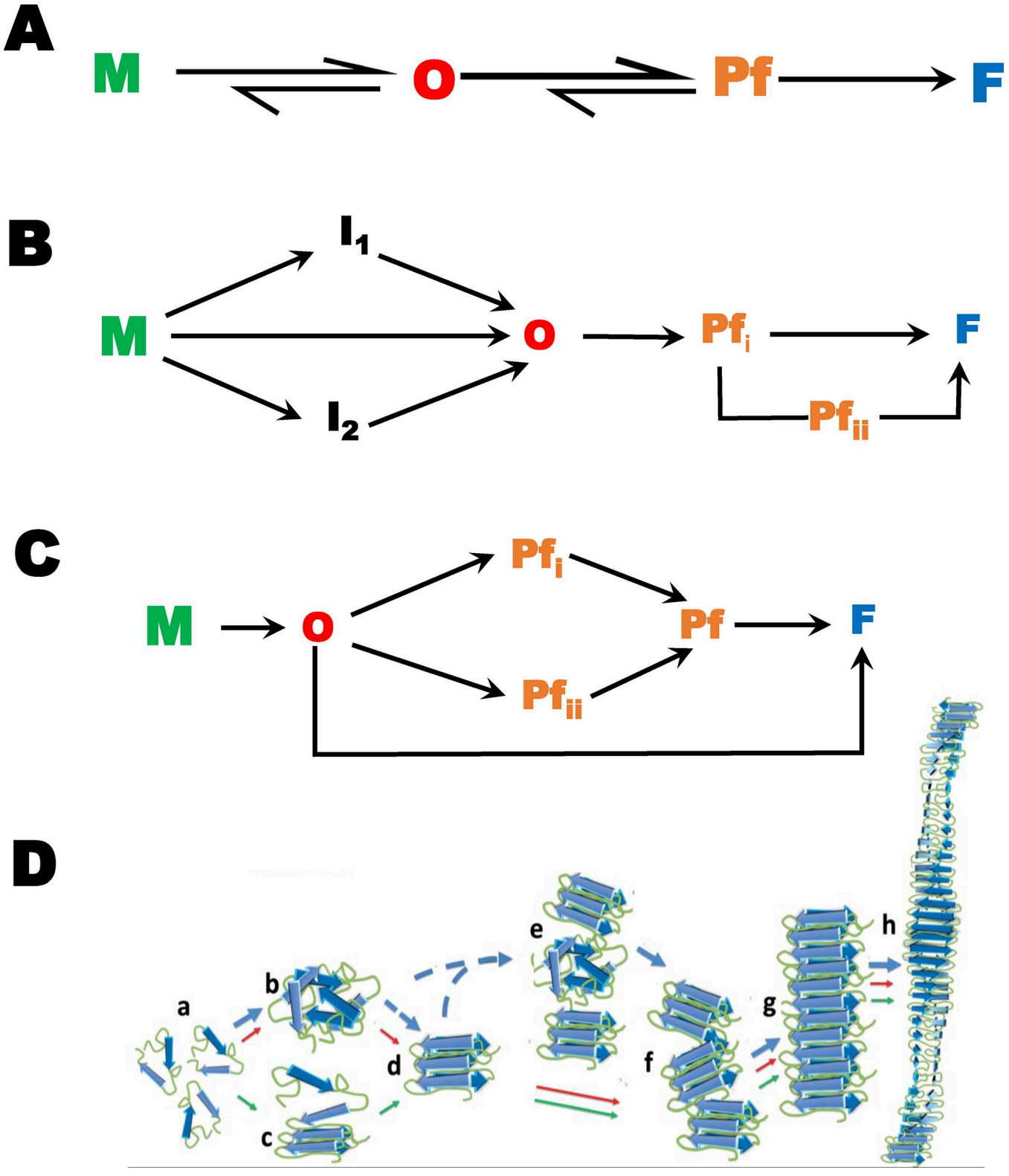
Scheme 1. Trajectories of Amyloid Aggregation: (A) Linear Trajectory Showing Reversibility among Species, (B) Complex Trajectory with Multiple Pathways, (C) Another Complex Trajectory with Multiple Pathways, and (D) Potential Pathways by Which Soluble Aβ Converts into Oligomers and Protofibrils before Forming Mature Fibrils[a]



Adapted from Lipiec et al. (2018) *Angew. Chem., Int. Ed.* 57, 8519−8524.[1] [a]M: Monomer. I: Intermediate and its subtypes. O: Oligomer. Pf: Protofibril and its subtypes. F: Fibril.

based on topographical criteria. The microscopy images revealed a terrain rich in morphologically discrete species. Fibrils appeared as much smoother, thinner and more densely packed than the chain-like protofibrils and could be easily

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discriminated. An oligomer stuck to a fibril was also resolved as such.[1]

Chemical mapping was performed using standard vibrational parameters associated with proteins. Ordered and disordered β-sheets were detected in both protofibrils and oligomers. By combining knowledge about the particle size and shape, the degree of β-sheet order, whether the sheets were parallel or antiparallel, and the degree of secondary structural elements within a conformer (its chemical signature), the authors were able to construct the pathway by which monomeric Aβ sequentially transforms into mature fibrils.

Results from the study demonstrated that soluble, monomeric Aβ peptides merge to form oligomeric particles with loosely aggregated strands and disordered β-sheets.[1] This intermediate then undergoes partial structural reorganization to form well-defined oligomers containing a parallel distribution of β-sheets. The well-defined oligomers then coalesce to form protofibrillar intermediates containing a disordered and antiparallel distribution of β-sheets. The segmentally disordered protofibrillar intermediates undergo a partial structural reorganization to form other types of protofibrils with a parallel distribution of β-sheets. Finally, the ordering of the protofibril core in this population results in securing a classical protofibrillar structure before advancing to mature fibrils.

The inadequacy in resolving the innate heterogeneity in the protein folding and misfolding reactions not only generates false road maps and hypothetical pathways but also remains a principal causal in the failure to make therapeutic inroads into amyloid pathologies and neurodegenerative disorders in particular.[5] TERS has clearly demonstrated its potential to directly detect and resolve amyloid structures with nanoscale resolution and determine the aggregation and aggregation-inhibition pathways of amyloid proteins.[1,2] The outcomes from Zenobi's work are paradigm shifting. It is now clear that the exemplar amyloid Aβ morph into mature fibrils by populating mixed oligomeric and protofibrillar conformers. Furthermore, the fact that loosely structured oligomers can convert into protofibrils via two mechanisms results in the transformation of a previously generally postulated unidimensional (linear) pathway into a two-dimensional network of interactive routes to a toxic conformation.

Future studies unravelling the susceptibility of on-pathway intermediates, oligomers, protofibrils, and the interactions between them to extrinsic conditions, mutations, and small-molecules and the impact on neurotoxicity are clearly on the horizon, as are efforts that will undoubtedly be geared to characterizing the amyloidogenic pathways of proteins, such as mHTT, α-synuclein, and tau.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Lipiec, E., Perez-Guaita, D., Kaderli, J., Wood, B. R., and Zenobi, R. (2018) Direct Nanospectroscopic Verification of the Amyloid Aggregation Pathway. *Angew. Chem., Int. Ed. 57*, 8519−8524.

(2) Deckert-Gaudig, T., and Deckert, V. (2016) High resolution spectroscopy reveals fibrillation inhibition pathways of insulin. *Sci. Rep. 6*, 39622.

(3) D'Andrea, C., Foti, A., Cottat, M., Banchelli, M., Capitini, C., Barreca, F., Canale, C., de Angelis, M., Relini, A., Maragò, O. M., Pini, R., Chiti, F., Gucciardi, P. G., and Matteini, P. (2018) Nanoscale Discrimination between Toxic and Nontoxic Protein Misfolded Oligomers with Tip-Enhanced Raman Spectroscopy. *Small 14*, 1800890.

(4) Paulite, M., Blum, C., Schmid, T., Opilik, L., Eyer, K., Walker, G. C., and Zenobi, R. (2013) Full Spectroscopic Tip-Enhanced Raman Imaging of Single Nanotapes Formed from β-Amyloid(1−40) Peptide Fragments. *ACS Nano 7*, 911−920.

(5) Kabiraj, P., Marin, J. E., Varela-Ramirez, A., and Narayan, M. (2016) An 11-mer Amyloid Beta Peptide Fragment Provokes Chemical Mutations and Parkinsonian Biomarker sAggregation in Dopaminergic Cells: A Novel Road Map for "Transfected" Parkinson's. *ACS Chem. Neurosci. 7*, 1519−1530.

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